Multiple component networks support working memory in prefrontal cortex

David A. Markowitz\textsuperscript{a}, Clayton E. Curtis\textsuperscript{a, b}, and Bijan Pesaran\textsuperscript{a, 1}

\textsuperscript{a}Center for Neural Science, New York University, New York, NY 10003; and \textsuperscript{b}Department of Psychology, New York University, New York, NY 10003

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Lateral prefrontal cortex (PFC) is regarded as the hub of the brain’s working memory (WM) system, but it remains unclear whether WM is supported by a single distributed network or multiple specialized network components in this region. To investigate this problem, we recorded from neurons in PFC while monkeys made delayed eye movements guided by memory or vision. We show that neuronal responses during these tasks map to three anatomically specific modes of persistent activity. The first two modes encode early and late forms of information storage, whereas the third mode encodes response preparation. Neurons that reflect these modes are concentrated at different anatomical locations in PFC and exhibit distinct patterns of coordinated firing rates and spike timing during WM, consistent with distinct networks. These findings support multiple component models of WM and consequently predict distinct failures that could contribute to neurologic dysfunction.

High-level cognition depends on the ability to translate stored information about recent experience into a behaviorally appropriate response, an ability known as working memory (WM). WM relies on a storage process that actively maintains information and a control process that manipulates stored information to support the selection and preparation of a contingent response (1–3). The neural mechanisms that support WM involve networks that are broadly distributed throughout the brain (4–7) and rely heavily on the prefrontal cortex (PFC) for normal operation (6–9). However, the degree to which WM is supported by a single distributed network or multiple specialized network components in PFC remains unclear (6, 10, 11), hindering progress in the search for neurocognitive therapies to treat disorders of cognition (12).

Persistent spiking activity is commonly thought to reflect the mechanistic basis of WM in PFC (13–16). This activity manifests in different ways, including time-varying neuronal responses that decay, ramp up, or are stable in time during memory delays. Although such a diversity of responses could reflect distinct modes of persistent activity, it has long been a standard practice to treat all such a diversity of responses could reflect distinct modes of persistent activity. Although this reasoning does not exclude the possibility that the visual delay also support these three modes are organized with distinct spatio-temporal profiles in PFC. These results suggest that information storage and the preparation of contingent responses are supported by functionally specialized networks in PFC.

### Results

To test whether storage and response preparation are supported by different modes of persistent activity during WM, we mapped neural activity with chronically implanted movable multielectrode arrays placed over the right prearcuate gyrus of lateral PFC in two monkeys (20). During each experimental session, each monkey performed an ODR task with randomly interleaved trials of memory-guided (mODR) and visually guided (vODR) saccades to one of eight isoeccentric targets (Fig. 1A) (21). Over the course of 41 sessions in monkey A and 43 sessions in monkey S, we slowly advanced the electrodes to isolate and record from 746 units (384 in monkey A, 362 in monkey S) at multiple depths extending 2.5 mm below the cortical surface. After the completion of all recordings, we registered the depths measured on each electrode to the cortical surface, using an iterative algorithm (see Experimental Procedures).

We hypothesized that interleaving memory-guided and visually guided trials should reveal multiple distinct storage and response preparation-related modes of persistent activity. Our first prediction is that storage modes should be revealed by differences in activity across the memory and visual delay conditions that arise from differences in how information is maintained during each task (Fig. 1B). A memory–visual comparison tests a critical assumption made by previous studies: that a memory delay is sufficient to identify neurons that reflect storage, and by extension, that a visual delay involves the same storage process as a memory delay. Note that this reasoning does not exclude the possibility that the visual delay also

### Significance

Human cognition depends on the brain’s ability to remember and manipulate information about recent experience. Although this working memory ability has been linked with the persistent spiking activity of neurons in prefrontal cortex (PFC), the population-scale organization of working memory in this region remains poorly understood. Here we provide the first population-scale map, to our knowledge, of persistent activity across multiple depths and topographic regions of PFC in two monkeys performing a delayed eye movement task. We show that working memory is supported by three functionally specialized networks with different anatomical localizations, and not by a functionally homogeneous network, as commonly believed. These findings reveal specific circuit-level targets for studying the causes of working memory dysfunction in many psychiatric disorders.

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1To whom correspondence should be addressed. Email: bijan@nyu.edu.

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Experimental design for testing the distinct mode hypothesis. (A) ODR paradigm with interleaved mODR and vODR trials. (B and C) Predicted responses of neurons that encode stored information and prepared responses as a function of (B) delay type and (C) reaction time after a memory delay. (D) Preferred target spike rasters and peri-stimulus time histograms (PSTHs) for two spatially tuned units that fire persistently during the memory delay. mODR traces in red, vODR traces in blue. Black bar denotes Cue interval. Event alignment labels: C (Cue), G (Go), S (Saccade). (i) Candidate storage unit. (ii) Candidate response unit. (E) Two modes of activity that were identified by principal component analysis in the full population of 746 recorded units. (i) Candidate storage mode. (ii) Candidate response mode. Axis labels as in D.

Fig. 1. Experimental design for testing the distinct mode hypothesis. (A) ODR paradigm with interleaved mODR and vODR trials. (B and C) Predicted responses of neurons that encode stored information and prepared responses as a function of (B) delay type and (C) reaction time after a memory delay. (D) Preferred target spike rasters and peri-stimulus time histograms (PSTHs) for two spatially tuned units that fire persistently during the memory delay. mODR traces in red, vODR traces in blue. Black bar denotes Cue interval. Event alignment labels: C (Cue), G (Go), S (Saccade). (i) Candidate storage unit. (ii) Candidate response unit. (E) Two modes of activity that were identified by principal component analysis in the full population of 746 recorded units. (i) Candidate storage mode. (ii) Candidate response mode. Axis labels as in D.

Involves a storage process, and that this reasoning only predicts that storage differs across the memory and visual delays. In contrast, we reasoned that the modes reflecting the selection and preparation of the response should not differ across the memory–visual comparison. We predict that the activity of neurons related to the response will be elevated equally during both memory and visual delay periods, because the same eye movement is selected and prepared under both conditions. Furthermore, we predict that the storage and response processes should differ in their relationships to movement reaction time (RT) after a memory delay (Fig. 1C). Specifically, the activity of neurons that encode prepared responses will covary with future RT, whereas the activity of storage neurons will not, because only the response mode, which reflects the manipulation of information to generate the response, influences the timing of behavior after the delay. With these predictions in mind, we examined the neural responses.

We first examined individual neuron responses to test whether both storage and response activities are present in PFC. We found that many PFC neurons responded with different activity patterns during the memory and visual delays (Fig. 1 D, i), consistent with a role in a memory storage process. Others responded equally during the delay periods of both tasks (Fig. 1 D, ii), consistent with a role in preparing the response. Finally, a third population responded strongly during the extended presentation of preferred visual stimuli and subsequent visual delay, but only briefly during the memory delay, consistent with a role in a cue encoding and early storage process. These task-selective responses were present in each monkey individually, and typically emerged in response to preferred target locations only (SI Appendix, Fig. S1).

Although the presence of memory delay-specific responses suggests that storage and response preparation map to distinct modes of activity, individual examples are not sufficient to draw this conclusion. To more rigorously test whether distinct single-unit responses are representative of distinct modes at the population level, we studied population dynamics using a dimensionality reduction approach. We used principal component analysis to identify eigenmodes of population activity that compactly describe the responses of all 746 isolated neurons, including those without clear spatial tuning or task selectivity, across all eight targets during both tasks (see Experimental Procedures). This revealed modes with delay activity that qualitatively resembled the task-selective single unit responses described earlier, including putative storage-related (Fig. 1 E, i) and response-related (Fig. 1 E, ii) modes and a visually driven mode with decaying activity during the memory delay (SI Appendix, Fig. S2A).

To determine whether the putative storage- and response-related modes of population activity are associated with functionally distinct networks, we further examined the activity of individual PFC neurons. In general, single unit responses reflect a mixture of persistent modes across all phases of the task (SI Appendix, Fig. S2B), arguing against the existence of functionally specialized neurons. However, many responses are clearly dominated by a single task-selective mode during the late delay, just before the Go command, as illustrated in Fig. 1D. If different unit responses reflect activity in different WM modes, then we reasoned it should be possible to study and compare the properties of different modes by operationally classifying single-unit responses by their dominant mode during the late delay.

To support this analysis, we identified units that persistently encoded spatial information during the late delay and then classified them by their task selectivity during this epoch. We focused on positively tuned regular-spiking units in this study (n = 365; SI Appendix, Fig. S3) because neurons of this type are believed to play a fundamental role in the maintenance of persistent activity states in PFC (15, 22). To classify neurons, first we quantified each unit’s activity in the memory-selective population ramped up during brief periods, consistent with a role in a cue encoding and early storage process, suggesting a weakening role in storage over time. In contrast, activity in the memory-selective population ramped up during the memory delay, but not the visual delay, consistent with a late...
storage process (Fig. 2A, ii). The number of spatially tuned units in this population increased from 67 to 93 during the transition from the early to late memory delay, suggesting a strengthening role in storage over time. We confirmed that these two populations capture distinct storage-related modes of activity at the population level by performing a targeted dimensionality reduction analysis of all 746 recorded units (see Experimental Procedures). Targeted dimensionality reduction is a constrained extension of principal component analysis that seeks to discover the dimensions of variability in data that are most closely linked with task variables (in this case, target location and whether or not memory storage was required during the delay). This analysis revealed two modes of task-related persistent activity that exhibited slowly decaying and slowly ramping activity during the memory delay, consistent with early and late storage modes (SI Appendix, Fig. S4). Finally, neural activity persisted in the nontask-selective population during both delays, consistent with response preparation (Fig. 2A, iii). These results demonstrate that persistently active units can be assigned to populations that broadly reflect three distinct modes of persistent activity in PFC, which may be linked to storage and response preparation.

If stored information and response preparation map to different modes of population activity, the second prediction made earlier is that the mode that encodes responses should encode the metrics of a prepared movement, whereas the two storage modes should not. If this is correct, response-related activity immediately before the Go command would covary with RT, whereas storage activity will not, as predicted by the distinct mode hypothesis. To test this prediction, we grouped memory trials by the fastest 50% and slowest 50% of saccades made by each monkey and then compared the preferred target responses of each neural population across RT conditions during the last 500 ms of the memory delay. We reasoned that activity during this presaccadic interval was most likely to reveal whether a population played a role in determining subsequent RTs. Spike rates did not differ across RT conditions in the early storage (Fig. 2B, i) or late storage (Fig. 2B, ii) populations (P > 0.05 permutation test). In contrast, delay activity in the response population was significantly lower during slow RT trials than during fast trials (Fig. 2B, iii; P < 0.001 permutation test). These results hold for each animal individually. A further comparison of delay activity across correct trials and infrequent saccade error trials (2.6% of trials; 15.4° median angle between cue location and saccade endpoint) revealed that only the response population showed a significant drop in activity before inaccurate saccades (SI Appendix, Fig. S5). Therefore, information encoded in the response network directly influences the timing and accuracy of planned behavior. Together, these results demonstrate that storing information and generating a response involve distinct modes of persistent activity in PFC.

The identification of storage and response preparation with different groups of neurons raises the possibility that these networks could map to distinct anatomical regions. Therefore, we investigated whether these circuits were randomly organized in PFC or whether they were functionally localized with distinct spatial signatures. Specifically, we used our database of recordings from chronically implanted movable electrode arrays to map the spatial organization of persistently active neurons within a 3D cortical volume. Although neurons from each WM network were broadly distributed throughout the sampled volume, the resulting brain maps revealed localized functional differences with substantial changes in the topography of the late storage and response networks (Fig. 3A and B). Neurons in the late storage network were concentrated in posterior PFC, proximal to area 8, whereas the response network was concentrated in anterior PFC, proximal to area 46. These unit classes occurred with significantly different likelihoods in the anterior half of the array (0.68 ± 0.04 SEM for response neurons vs. 0.25 ± 0.03 SEM for late storage neurons; P < 0.05, binomial exact test). Furthermore, late storage neurons were most likely to be recorded from superficial depths (Fig. 3C and D; 0.37 ± 0.06 SEM at 0.1 mm vs. 0.22 ± 0.05 SEM at 1.0 mm; P < 0.05, binomial exact test), whereas response neurons were highly biased toward deeper sites (0.73 ± 0.07 SEM at 1.3 mm vs. 0.50 ± 0.06 at 0.1 mm; P < 0.05, binomial exact test). The structure of these anatomical maps does not change after excluding data from electrodes that were suspected to have descended down sulcal banks. Together, these findings demonstrate that the storage and response preparation modes are spatially organized and not randomly dispersed in PFC.
thus conclude that WM is supported by multiple anatomically specific modes of persistent activity.

The clear functional and anatomical differences between the storage and response populations suggest they make up distinct networks. However, these populations could also reflect different aspects of a single, functionally integrated, distributed network that uses long-range interactions to coordinate three anatomically specific modes of activity. We tested this hypothesis by looking for evidence that persistent activity is coordinated across columns separated by ≥1.5 mm within each of the three populations, as expected for components of a large network with globally coordinated activity. We quantified “coordination” as the trial-to-trial correlated variability (“noise correlation”) between the firing rates of similarly tuned neurons that were recorded on different electrodes (see Experimental Procedures). We found that similarly tuned pairs within the early and late storage populations exhibited positive noise correlations during the memory delay and zero noise correlation during the visual delay (Fig. 4A), consistent with memory delay-specific coordination across columns. In contrast, similarly tuned neuron pairs in the response network exhibited zero noise correlation during both memory and visual delays, indicating that distributed coordination is unlikely to support persistence within this population. All correlations were independent of firing rate and were zero during the memory delay in both storage and response pairs with large spatial tuning differences (Fig. 4B). These results indicate that coordinated cross-columnar interactions can support persistence in the two storage populations, but cannot support persistence in the response population. Therefore, the storage and response populations reflect anatomically distinct networks that coordinate their activity over different spatial scales.

Although the generation of a contingent response from a stored memory is clearly required for the mODR task, the lack of a clear link between the firing rates of the storage and response networks makes it unclear whether or how the storage mode of population activity influences the memory-guided response. For example, if the response mode changes its functional significance across the visual and memory delays, such that the response network supports all maintenance and manipulation functions, then the late storage mode may not be involved in the generation of a memory-guided response. To study this problem, we tested the hypothesis that the timing of action potentials in all three populations is coordinated in relation to the timing of a prepared response, which is one possible signature of networks that collectively support memory-guided behavior (24). To quantify “coordination” across each population, we estimated spike-field coherence between spikes and local field potentials recorded on separate electrodes within each network during the last 1 s of the memory delay on preferred stimulus trials, and then compared spike-field coherence across fast and slow RT trials (see Experimental Procedures). In support of the temporal coordination hypothesis, spike-field coherence in the 14–30-Hz band was strongest during slow RT trials among all three populations of early storage (Fig. 4C), late storage (Fig. 4D), and response neurons (Fig. 4E) (P < 0.05, permutation test). This coherence
persisted throughout the delay (SI Appendix, Fig. S6) and was significantly weaker during fast RT trials, indicating a potential role for coherent activity in response selection. In further support of the hypothesis that multiple networks are coordinated during memory-guided behavior, neurons in all three populations displayed distinct patterns of temporal coordination during memory delays on slow RT trials. The preferred spike phases at 20 Hz were precisely organized (Fig. 4F), such that spikes occurred in a sequence of phases that were earliest in the early storage population and latest in the late storage population. This relative phase code establishes the involvement of the storage and response networks in memory-guided behavior and reveals that PFC maintains a strict temporal separation between the neural codes for stored information and prepared responses.

**Discussion**

Here we present several convergent lines of evidence to support models of WM that posit distinct component networks (1, 3, 9). Using a simple behavioral manipulation, we reveal a previously unknown distinction between storage- and response-related modes of persistent activity in the brain. The storage and response populations shown here display distinct functions, are patterned differently in the anatomical domain, and exhibit distinct patterns of firing rate and spike timing coordination during WM. Together, these findings demonstrate that anatomically specific modes of persistent activity correspond to distinct storage and response networks in PFC.

We identify the response network with a preparatory process because response population delay activity is linked with reaction time and saccade accuracy after the Go command. Both forms of behavioral variability are internally directed by the monkey in our experiments and are not instructed by external cues, making them good indicators of the monkey's internal state of preparation. This method of identifying internally directed responses during a prosaccade task is complementary to previous task designs that explicitly separated cue location and the direction of the impending response (25–27). Those studies pooled activity across the entire delay (without distinguishing between early or late delay epochs) and found that only 13–30% of persistently tuned units encode a prepared response. However, subsequent work has shown that the vector of PFC population activity gradually rotates during the delay period from the cue direction to the sac- cade direction (28), indicating that a single statistic may be insufficient to capture nonstationary responses in this area. Our work supports this view by demonstrating that a majority of units with late delay tuning are linked with response preparation (200/365 units, or 55%). It should be noted, however, that the response population identified here might not exclusively mediate response preparation and may also be involved in response selection. The response network could also mediate an additional component of the storage process that is indistinguishable from response-related activity, using our task design. Therefore, although we have shown that the early and late storage populations are distinct from the response network, the possibility remains that other aspects of the storage process are supported by the response network in PFC.

Furthermore, although we ascribe a memory storage process to the late storage network, in principle, this activity could also reflect a spatial attention process that in turn supports memory. In some cases, spatial WM may depend on the sustained allocation of attention to the spatial location of the memorized cue (29, 30). Although human fMRI studies disagree on whether attention mechanisms account for activity in PFC during WM maintenance (30, 31), at the cellular level, neurons in monkey PFC can encode stored locations and attended locations, and some even encode both (32). Although our study was not designed to distinguish between the storage and attention-related cognitive mechanisms, it firmly establishes a mode of population activity in PFC that encodes locations only when visual cues have been extinguished during the memory delay. Simple attention cannot explain this finding because one would expect that during the delay period, attention would be directed toward both the visible and memorized target. In contrast, theories of WM have always incorporated attention in one way or another (2). For instance, the storage of WM representations may be maintained through sustained attention to internal representations of the memoranda. Future work in this area is needed to clarify the role attention may play in WM processes.

In the anatomical domain, the storage and response networks are most prevalent at different topographic locations and depths of PFC. This functional topography is striking, in part because a previous study using the same task design and acute recording techniques did not find evidence for a specialized storage network in primate PFC (33). That study focused on recording sites that were mostly anterior to where we have observed the late storage network, however, indicating that a difference of only a few millimeters in recording location was critical to finding this network in PFC. Our results are also striking because the depth dependencies of the late storage and response networks are broadly consistent with tracing studies that have observed both highly recurrent cross-columnar interactions in superficial PFC (34), suggestive of a storage function, and projections from deep layers of PFC to subcortical areas involved in oculomotor control (35, 36), suggestive of a response function. Furthermore, the segregation of storage and response networks along the anteroposterior axis provides important physiologic support for previous human imaging results that suggest anterior regions of frontal cortex may be specialized for the preparation of goaldirected responses (10). A synthesis of these findings with previous studies of oculomotor control suggests that after the Go command is received, control of saccadic eye movements likely shifts from anterior to posterior locations in the frontal eye field (FEF), through the activity of classically identified “visuomovement” and “movement” neurons (37). We were unable to test this hypothesis in our data because we targeted gyral sites that were mostly anterior to FEF, and we did not perform microstimulation to identify FEF neurons to avoid damage to the recording electrodes and changes in how the measured responses varied with depth. Therefore, understanding how neurons in the distributed PFC storage and response preparation networks overlap with FEF is another important goal for future work.

In the temporal domain, the precisely organized phase lag between action potentials from the storage and response networks could implement a multiplexed code for memoranda and their contingent responses. The selective implementation of this code during trials with slow RTs suggests spike timing may directly influence the timing of response selection during the delay. Moreover, this code demonstrates that the brain uses coherent activity to maintain a temporal separation between the neural codes for stored information and prepared responses during memory-guided behavior. This separation may be necessary when behavior depends on the relationship between both a past stimulus and a rule-based response, such as is required for memory-guided antisaccades. In this regard, phase coding in PFC may reflect a basic mechanism of flexible behavioral control during WM. Moreover, such a mechanism could be applied generically to organize distinct information streams in support of other “top-down” processes that evoke coherent activity, such as attention (38), motor coordination (39), and decision making (40).

The finding that memory-guided saccade errors are linked with a failure of response preparation and not storage (SI Appendix, Fig. S5) supports a long-suspected but previously unproven mode of WM failure in PFC (14, 17, 25). Our results build on the recent finding that saccade accuracy is parametrically linked with the level of persistent activity by spatially tuned neurons in PFC (19), through
our demonstration that inaccurate saccades are specifically linked with a change in response-related activity only. Furthermore, these results inform disease models by indicating that impairments of memory-guided behavior in neuropsychiatric disorders (41, 42) may arise from disrupted response preparation, rather than from disrupted storage, as commonly believed. This revised hypothesis is consistent with the physiological effects of antipsychomimetic agents that are commonly used in the treatment of schizophrenia, because D1 antagonist administration evokes persistent activity in two classes of neurons: those that exhibit ramping responses, consistent with the late storage network, and those that exhibit plateau responses, consistent with the response network (43). Therefore, it is plausible that pharmacologic agents used in the treatment of WM disorders act primarily by counteracting degraded activity in the response network, while simultaneously enhancing activity in an otherwise functional late storage network. Future work will need to test the link between dopamine receptor type and WM component-specific activity in PFC.

**Experimental Procedures**

We obtained neuronal recordings from across the depths of the arcuate gyrus of two macaque monkeys (Macaca mulatta) while the animals performed either a memory-guided or a visually guided ODR task on randomly interleaved trials. We then analyzed the recordings to determine whether networks recruited by WM contain components with different functions. All surgical and animal care procedures were approved by the New York University Animal Care and Use Committee and were performed in accordance with the National Institutes of Health guidelines for care and use of laboratory animals (44). Detailed methods can be found in the SI Appendix, Experimental Procedures.

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44. Committee on Care and Use of Laboratory Animals (1996) Guide for the Care and Use of Laboratory Animals (Natl Inst Health, Bethesda), DHHS Publ No (NIH) 85-23.